obtained bacterial cells are disrupted by a French press or the like and centrifuged. The obtained supernatant fraction is fractionated by using a MonoQ (produced by Pharmacia) column, and the target fraction is concentrated. The fraction is fractionated by using a Superose 6 column, and the target fraction is dialyzed and fractionated by using a MonoS column to perform the purification.

An example of purification of the Sso7d protein obtained from *Sulfolobus solfataricus* is shown in the working examples mentioned hereinafter. It can also be purified from bacterial cells such as *Escherichia coli* cells in which the protein is highly produced thanks to a Sso7d protein gene introduced by a gene recombination technique.

The hybridization can be performed in the same manner as the usual nucleic acid hybridization, except that the hybridization is performed in the presence of a double-stranded DNA-binding protein. Specifically the hybridization can be performed as follows. First reducing agents such as dithiothreitol(DTT) and 2-mercaptoethanol, bovine serum albumin(BSA) and skim milk which prevent protein from non-specific ~~biding~~ binding to vessel and stabilize protein are added, and further protein accessory factors such as magnesium chloride($MgCl_2$), salt-condensation regulators such as sodium chloride(NaCl) and potassium chloride(KCl) and so forth

are added as required into buffer such as Tris buffer, phosphate buffer, citric acid ~~byffer~~ buffer, TES buffer, HEPES buffer or the like. The double-stranded DNA ~~biding~~ binding protein is then added to the solution. In this hybridization solution, the aforementioned oligonucleotide-immobilized nylon membrane (DNA (macro)array) and a labeled sample DNA are hybridized preferably for 1-20 minutes within a range of 40-120°C. When Sso7d is used as the double-~~staranded~~ stranded DNA binding protein, to a Tris buffer, 0.1-100 mM of DTT, 0.1-100 mM of $MgCl_2$ and 1-100 $\mu g/\mu l$ of BSA (all of the concentrations are final concentrations) are added, and the Sso7d protein is added to the solution within a range of 0.001-10%. In this hybridization solution, the oligonucleotide-immobilized nylon membrane (DNA (macro)array) and a labeled sample DNA are hybridized preferably for 1-15 minutes within a range of 40-70°C.

After the hybridization reaction, the membrane was washed with buffer such as Tris buffer, phosphate buffer, citric acid ~~byffer~~ buffer, TES buffer, HEPES buffer or the like for 1-10 minutes within a range of 10-50°C, and then dried. In this case, adding a small amount of surfactant such as sodium dodecyl sulfate (SDS) is preferable because it can prevent from non-specific binding and step down ~~backgraound~~ background. When Sso7d is used as the double-~~staranded~~ stranded DNA binding protein, after the hybridization reaction, it is preferable that the

membrane should be washed with citric acid buffer such as SSC, more preferably buffer added with SDS within a range of 0.001-0.05% as required to SSC, for 3-10 minutes within a range of 10-40 °C, and then air-dried. As for the hybridization signal, radiation dose or the like of each dot on the dried nylon membrane can be measured by autoradiography etc. to calculate the hybridization strength.

Detection of hybridization can be measured by a method suitable for each of various labeling methods. In the gene expression monitoring method, it is preferable to use fluorescent labeling because this method enables simultaneous detection of the expression strength for a plurality of sample nucleic acids by labeling them with a plurality of fluorescent dyes each having a different detection wavelength.

Examples of the application of the nucleotide sequence analysis according to the present invention include: DNA nucleotide sequence determination [Genomics, Vol. 4, p. 114-128 (1989)], diagnosis of infectious and genetic diseases etc. [J.G. Hacia et al., Nature Genetics, 14, 441-447 (1996)], mapping of giant genome DNA [BioTechniques, vol.17, No.2, p.328-336 (1994)], single-nucleotidepolymorphisms (SNPs) [Wang et al. Science, Vol. 280, p. 1077-1082 (1998)], the amplification of genes or analysis of deleted regions by

genes of a genome bank, the location of each clone on the genome can be determined.

Further, a test kit for using to perform the aforementioned gene analysis can be prepared by using a double-stranded DNA-binding protein. Such a test kit is constituted by components similar to those of ordinary test kits for gene analysis utilizing hybridization except for the use of the double-stranded DNA-binding protein. That is, the test kit of the present invention comprises at least a double-stranded DNA-binding protein and, as optional components, washing solution, diluent, hybridization solution and so forth.

EXAMPLES

The present invention will be explained more specifically with reference to the following examples. However, the present invention is no way limited by these examples.

Example 1

(A) Synthesis of probe DNA and sample DNA

The oligonucleotides shown in Table 1 were synthesized by using a DNA synthesizer (apparatus name: Expedite 8909) manufactured by PerSeptive Biosystems.

Immobilization of the oligonucleotides may be facilitated by modifying their 5' or 3' ends with 5'

The oligonucleotides (1) and (2), and the sample DNA (3) were concentrated to dryness, and then suspended in a 0.5 M sodium hydrogencarbonate buffer (pH 8.4) for (1) and (2), or TE buffer for (3), and they are quantified based on absorbance at 260 nm and adjusted to 1 nmol/μl.

(B) Immobilization of probe DNA (preparation of DNA array)

Immobilization of the oligonucleotides on a substrate was attained by bonding amino groups of the amino-modified oligonucleotides to a nylon membrane containing anionic carboxyl groups on its surface at a high density through amide bonds, as described below.

A Biodyne C (~~trade mark~~ trademark, produced by Pall) membrane was rinsed with 0.1 N HCl to acidify it, and immersed in 20% EDC (1-ethyl-3-dimethylaminopropylcarbodiimide hydrochloride) at room temperature for 15-30 minutes. The membrane was lightly rinsed with deionized water and 0.5 M sodium hydrogencarbonate buffer (pH 8.4), then mounted on a dot blot apparatus (produced by Bio-Rad) and allowed to react with the amino-modified oligonucleotide (1) or (2), which was suspended in a 0.5 M sodium hydrogencarbonate buffer (pH 8.4), at room temperature for 15 minutes.

The membrane was washed with TBS (Tris-buffered saline)/0.1% Tween-20, then treated with 0.1 N NaOH for

25°C for 5 minutes, washed three times with 0.2% SDS at 25°C (room temperature) for 1 minute, finally washed with sterilized water at 25°C for 1 minute, and air-dried.

(C) Hybridization reaction

70 μg/ml of the Sso7d protein and 100 pmol/ml of the sample DNA (8) were added to Buffer E (20 mM Tris buffer, pH 7.5, 2 mM DTT, 5 mM $MgCl_2$, 10 μg/μl of BSA), and the aforementioned oligonucleotide-immobilized slide glass (DNA microarray) and the fluorescently labeled sample DNA were allowed to hybridize at 60°C for 6 minutes in the solution. As a control, hybridization reaction was also performed in the same manner without adding the Sso7d protein.

After the hybridization reaction, the membrane was washed with 1 x SSC/0.03% SDS buffer at 25°C for 5 minutes, rinsed with 0.2 x SSC, and then further rinsed with 0.5 x SSC. After the washing solution was removed by centrifugation, the slide glass was air-dried.

The hybridization signal was evaluated by measuring amount of fluorescent dye of each spot on the air-dried slide glass by using ScanArray 3000 produced by ~~Genaral~~ General Scanning to calculate the hybridization signal strength. The results are shown in Table 5. The results are represented with relative values based on the hybridization signal strength of the probe DNA (4)

obtained with the addition of Sso7d, which is taken as 100.